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L8 with L3

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<u>L9</u>	L8 with L3	25	<u>L9</u>
<u>L8</u>	hematopoietic or stem or bone marrow	236741	<u>L8</u>
<u>L7</u>	L6 with L3	3	<u>L7</u>
<u>L6</u>	polycationic or polybrene	5488	<u>L6</u>
<u>L5</u>	L4 and L3	77	<u>L5</u>
<u>L4</u>	not polybrene	27312467	<u>L4</u>
<u>L3</u>	L2 with L1	107	<u>L3</u>
<u>L2</u>	heparin or fibronectin	30569	<u>L2</u>
<u>L1</u>	retrovir\$	24122	<u>L1</u>

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File: USPT

Mar 7, 2000

US-PAT-NO: 6033907

DOCUMENT-IDENTIFIER: US 6033907 A

TITLE: Enhanced virus-mediated DNA transfer

DATE-ISSUED: March 7, 2000

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Williams; David A.	Indianapolis	IN		

US-CL-CURRENT: 435/325; 435/244, 435/320.1, 435/455, 435/456, 435/69.1, 536/23.1

## CLAIMS:

What is claimed is:

1. A method for obtaining a transduced population of viable mammalian cells by a retrovirus, comprising:

infecting the viable mammalian cells with a retrovirus in the presence of an effective immobilized amount of substantially pure fibronectin, substantially pure fibronectin fragments, or a mixture thereof, to increase the efficiency of transduction of the viable mammalian cells by the retrovirus, said infecting being conducted in a medium essentially free from a polycationic agent which increases the efficiency of transduction of the viable mammalian cells by the retrovirus in co-culture, but which agent reduces the efficiency of transduction of the cells by the retrovirus in the presence of said substantially pure fibronectin, substantially pure fibronectin fragments, or mixture thereof.

2. The method of claim 1 wherein the cells comprise hematopoietic stem cells.

3. The method of claim 1, wherein said cells are human cells.

4. A method for obtaining a transduced population of viable mammalian cells by a retrovirus, comprising:

infecting the viable mammalian cells with a retrovirus in the presence of an effective immobilized amount of substantially pure fibronectin, substantially pure fibronectin fragments, or a mixture thereof, to increase the efficiency of transduction of the cells by the retrovirus, said infecting being conducted in a medium essentially free from a polycationic agent which increases the efficiency of transduction of the viable mammalian cells by the retrovirus in co-culture, but which polycationic agent reduces the efficiency of transduction of the viable mammalian cells by the retrovirus in the presence of said substantially pure fibronectin, substantially pure fibronectin fragments, or mixture thereof, said infecting forming a population of viable mammalian cells transduced at an efficiency greater than that which would be achieved in the presence of said polycationic agent.

5. The method of claim 4 wherein said infecting is conducted in a medium free

from retroviral co-producer cells.

6. The method of claim 4, wherein the immobilized fibronectin, fibronectin fragments, or mixture thereof contains an amino acid sequence which provides the cell-binding activity of the CS-1 domain and an amino acid sequence which provides the retrovirus binding activity of the Heparin-II domain.

7. The method of claim 6, wherein the amino acid sequence which provides the retrovirus binding activity of the Heparin-II domain includes the amino acid sequence of SEQ ID NO:1.

8. The method of claim 6, wherein the amino acid sequence which provides the cell-binding activity of the CS-1 domain includes the amino acid sequence of SEQ ID NO:2.

9. A method for obtaining a transduced population of viable hematopoietic cells, comprising:

providing an in vitro population of hematopoietic cells;

infecting the hematopoietic cells with a retrovirus in the presence of substantially pure fibronectin, substantially pure fibronectin fragments, or a mixture thereof, said infecting being conducted in a medium essentially free from a polycationic agent which increases the efficiency of transduction of the hematopoietic cells by the retrovirus in co-culture, but which polycationic agent reduces the efficiency of transduction of the hematopoietic cells by the retrovirus in the presence of said substantially pure fibronectin, substantially pure fibronectin fragments, or mixture thereof.

10. The method of claim 9, wherein the immobilized fibronectin, fibronectin fragments, or mixture thereof contains an amino acid sequence which provides the cell-binding activity of the CS-1 domain and an amino acid sequence which provides the retrovirus binding activity of the Heparin-II domain.

11. The method of claim 10, wherein the amino acid sequence which provides the retrovirus binding activity of the Heparin-II domain includes the amino acid sequence of SEQ ID NO:1.

12. The method of claim 10, wherein the amino acid sequence which provides the cell-binding activity of the CS-1 domain includes the amino acid sequence of SEQ ID NO:2.

13. The method of claim 10, wherein the amino acid sequence which provides the retrovirus binding activity of the Heparin-II domain includes the amino acid sequence of SEQ ID NO:1, and wherein the amino acid sequence which provides the cell-binding activity of the CS-1 domain includes the amino acid sequence of SEQ ID NO:2.

14. A method for obtaining a transduced population of viable mammalian cells by a retrovirus, comprising:

infecting the viable mammalian cells with a retrovirus in the presence of an effective immobilized amount of polypeptide comprising a first amino acid sequence of SEQ ID NO:1, and a second amino acid sequence of SEQ ID NO:2, said infecting being conducted in a medium essentially free from a polycationic agent which increases the efficiency of transduction of the viable mammalian cells by the retrovirus in co-culture, but which polycationic agent reduces the efficiency of transduction of the viable mammalian cells by the retrovirus in the presence of said polypeptide, said infecting forming a population of viable mammalian cells transduced at an efficiency greater than that which would be achieved in the presence of said polycationic agent.

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File: USPT

Nov 11, 1997

US-PAT-NO: 5686278

DOCUMENT-IDENTIFIER: US 5686278 A

TITLE: Methods for enhanced retrovirus-mediated gene transfer

DATE-ISSUED: November 11, 1997

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Williams; David A.	Indianapolis	IN		
Patel; Vikram P.	Olney	MD		

US-CL-CURRENT: 435/456; 435/372

## CLAIMS:

What is claimed is:

1. A method for increasing the frequency of transduction of hematopoietic cells by a replication-defective recombinant retrovirus vector, comprising infecting hematopoietic cells with a replication-defective recombinant retrovirus vector in the presence of substantially pure fibronectin, substantially pure fibronectin fragments, or a mixture thereof, so as to increase the frequency of transduction of the hematopoietic cells by the retrovirus vector.
2. The method of claim 1 wherein the hematopoietic cells have a protein deficiency or abnormality and the recombinant retrovirus vector includes an exogenous gene encoding the protein.
3. The method of claim 1 wherein the cells are infected with the retrovirus vector in the presence of a fibronectin fragment containing the alternatively spliced CS-1 cell adhesion domain.
4. The method of claim 1 wherein the hematopoietic cells are a human hematopoietic cellular population including human stem cells.
5. The method of claim 2 wherein the exogenous gene is a gene encoding adenosine deaminase.
6. The method of claim 5 wherein the exogenous gene is a gene encoding human adenosine deaminase.
7. The method of claim 4 wherein the hematopoietic cells are adherent-negative, low density, mononuclear cells.
8. A method for producing transduced hematopoietic cells, comprising:  
  
infecting hematopoietic cells in culture with a replication-defective recombinant retrovirus in the presence of immobilized fibronectin, immobilized fibronectin fragments, or an immobilized mixture thereof, to produce transduced hematopoietic cells.

9. The method of claim 8 which includes harvesting the transduced hematopoietic cells.

10. The method of claim 8 wherein the hematopoietic cells have a protein deficiency or abnormality and the recombinant retrovirus vector includes an exogenous gene encoding the protein.

11. The method of claim 8 wherein the hematopoietic cells have an enzyme deficiency or abnormality and the exogenous gene is a gene encoding the enzyme.

12. The method of claim 11 wherein the hematopoietic cells are human hematopoietic cells having an enzyme deficiency or abnormality and the exogenous gene is a human gene encoding the enzyme.

13. The method of claim 11 wherein the hematopoietic cells have an adenosine deaminase deficiency and the exogenous gene encodes adenosine deaminase.

14. The method of claim 12 wherein the human hematopoietic cells have an adenosine deaminase deficiency and the exogenous gene encodes adenosine deaminase.

15. The method of claim 12 wherein the cells are infected with the retrovirus in the presence of an immobilized fibronectin fragment containing the alternatively spliced CS-1 cell adhesion domain.

16. The method of claim 15 wherein the hematopoietic cells are a human hematopoietic cellular population including human stem cells.

17. The method of claim 16 wherein the hematopoietic cells are adherent-negative, low density, mononuclear cells.

18. A method for improving retroviral-mediated gene transfer in hematopoietic cells, comprising conducting the retroviral-mediated gene transfer in the presence of immobilized fibronectin, immobilized fibronectin fragments or an immobilized mixture thereof.

19. The method of claim 18 wherein the hematopoietic cells are a mammalian hematopoietic cellular population including mammalian stem cells.

20. A composition comprising:

a viable hematopoietic cellular population transduced by retroviral-mediated gene transfer; and

immobilized fibronectin, immobilized fibronectin fragments, or an immobilized mixture thereof, in the presence of which said population has been transduced by the retroviral-mediated gene transfer;

said composition being free from virus-producing cells.

21. The composition claim 20 wherein the cellular population is a human hematopoietic cellular population including human stem cells.

22. The composition of claim 20 wherein the hematopoietic cellular population is comprised of adherent-negative, low-density mononuclear cells.